REMARKS

The Office Action dated March 25, 2008 and the references cited therein have been fully considered. Reconsideration of this application in view of the pending claims is respectfully requested in light of the following remarks.

Claims 15-29 are pending.

Claims 1-14 are canceled.

Claims 15-29 have been rejected.

Summary of the Invention

The invention pending in this application is directed to an assay, and cells useful in them, for detecting calcium channel modulators, and in particular antagonists, which are state dependent. As described in the Specification, voltage gated calcium channels inactivate as a function of membrane potential such that different conformational states of the channel are populated at different potentials. Since drug binding can be state dependent, being able to alter the membrane potential of cells via addition of potassium ions and an inward rectifier potassium channel, means that compounds can be identified which bind to different conformational states of the calcium channel. An assay that is amenable to being scaled up to high throughput is of significant use to the pharmaceutical industry, which is achieved by the present invention.

Applicants herein have engineered a cell line for use in the claimed assays that co-expresses a voltage gated calcium channel, an L-type calcium channel complex comprising alpha 1C, alpha 2-delta and beta 2a, and a Kir 2.3 inward K^+ rectifying channel. This cell line provides a functional calcium channel and a Kir 2.3 inward K^+ rectifying channel that has a set resting membrane potential.

Claim Rejections Under 35 U.S.C. §103

Claims 15-29 are rejected under 35 U.S.C. §103 (a) as being unpatentable over Harpold et al.(US 6,090,623) (Harpold), in view of Maher et al. (US 6,686,193) (Maher), and Bonini et al. (US 6,117, 990) (Bonini). Applicants respectfully traverse this rejection. The stated rejection cobbles together various elements or terms from each reference without consideration of the overall teaching of the reference and which in total does not teach or suggest the claimed invention.

The Office Action states that Harpold teaches a method of assaying for an antagonist with test compounds using a calcium channel comprising alpha 1c, alpha-2delta and beta 2a subunits. Harpold describes isolated DNA sequences encoding various human calcium channel subunits and splice variants, but it does not teach an isolated alpha 2-delta subunit (alpha 2 subunit and splice variants, but no delta spice variant), nor does it teach an isolated beta 2a subunit (only beta 2d). Similarly, while Harpold

describes the use of various combinations of subunits to form functional calcium channels, it does not teach an L-type calcium channel complex comprising alpha 1c, alpha 2-delta and beta 2a.

The Office Action states that Maher teaches an antagonist assay using multiple ion channels, including calcium channel and inward rectifier Kir 2.3, that can be monitored simultaneously in a 96 well plate. Maher is directed to a high throughput screening method using a system for electrical field stimulation (plate, electrodes and monitoring device) (EFS) and not to an assay for identifying state-dependent calcium channel antagonists using an engineered cell line that co-expresses an L-type calcium channel and an inward rectifying K^+ channel. Nor does it specifically describe any cell line that co-expresses a voltage gated calcium ion channel and an inward rectifier potassium channel, let alone the specific combination that Applicants used in the claimed assay. One would have to pick and choose from a lengthy list of sodium (Table 1), potassium (Table 2), chloride (Table 3) channels and subtypes, but would still come up deficient with respect to the specific calcium channels. Only general guidance and a prophetic description of an assay is given as to use of calcium ion channels (Col. 44-45). One of ordinary skill in the art would recognize and appreciate that expression of a functional calcium channel in combination with the inward rectifying K^+ channel would be essential to carrying out the assay claimed herein. Applicants have shown that the claimed assay, and cell lines, provide the expression and sensitivity to efficiently carry out the claimed assay (see Examples 2 and 3 of the Specification).

Even as to the potassium channels, Maher's description is general and only as to endogenous potassium channels, not ones that have been engineered to a set resting potential. One skilled in the art would appreciate that an endogenous cell line, and specifically a Kir 2.3 inward rectifying K^+ channel, is fundamentally different from one that has been engineered to set the resting membrane potential. With an endogenous ion channel it is unknown as to what membrane potential will be present, nor is the reproducibility of the ion channel guaranteed. In Example 2, Applicants have demonstrated that the assay, using the engineered cell line, can be used to identify antagonists for inhibition of K+ induced calcium influx. It should also be noted that while Maher describes the use in general (Col. 42-43) of inward-rectifier potassium channels for ion channels that can be assayed with their EFS system, it does not specifically identify a Kir 2.3 potassium channel as an inward-rectifying K+ channel.

The Office Action states that Bonini teaches using an inward rectifier potassium channel and intracellular calcium measurement using fluo-3 and FLIPR (Col. 6). Bonini is directed to isolated DNA sequences encoding a human SNORF1 receptor, a G-protein coupled receptor, and uses thereof. Applicants have not found where this reference makes any reference to the use of an inward rectifier potassium channel and an intracellular calcium measurement. The description in Col. 6 appears to be directed to the use of SNORF1 for use in measuring intracellular free calcium concentration. Nor does

the reference describe any means to identify an antagonist using an assay in which the cell membrane is depolarized.

Accordingly, it would not have been obvious to one of ordinary skill to modify the method of Harpold to incorporate the teachings of Maher as to multiple ion channels, including Kir 2.3 and calcium channel, in an assay with fluo-3 in a 96 well plate. Notwithstanding that one skilled in the art would not have been motivated to use the recombinant subunits of Harpold with the EFS system of Maher, there was no reasonable expectation of success in that the resulting assay is not the claimed invention. There is no teaching or suggestion of a membrane depolarization assay in which an antagonist is identified through the use of a cell line engineered to co-express a L-type calcium channel and a Kir 2.3 inward rectifying K+ channel.

Likewise, it would not have been obvious of ordinary skill in the art to modify the method of Harpold to incorporate the teachings of Bonini as to the use of FLIPR and fluo-3 to measure intracellular calcium concentration. One of ordinary skill in the art would have no reasonable expectation of success as to the assay resulting from this combination of references in that there is no teaching or suggestion as to the use of an inward rectifying K+ channel nor would one use a human SNORF1 receptor to identify an antagonist of a voltage-gated calcium ion channel.

CONDITIONAL PETITION

Applicants hereby make a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to affect this Conditional Petition.

CONCLUSION

In view of the foregoing amendments and remarks, it is seen that all grounds of rejection have been overcome and that Claims 15-29 are in proper condition for allowance. Accordingly, Applicants respectfully request that all rejections of record be withdrawn and that a Notice of Allowance be forwarded to the Applicants. An early Office Action to that effect is, therefore, earnestly solicited. The Examiner is invited to contact Applicants' Attorney at the telephone number given below, if such would expedite the allowance of this application.

Respectfully submitted,

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